

CLAIMS

We claim:

1. A method of detecting a first target sequence comprising a poly(A) sequence in a sample comprising:

5 a) hybridizing a first probe to said target sequence to form a first hybridization

complex, said first probe comprising:

i) an upstream universal priming site (UUP);

ii) an adapter sequence;

iii) a first target-specific sequence; and

iv) a downstream universal priming site (DUP);

10 wherein said poly(A) sequence remains single-stranded;

b) contacting said first hybridization complex with a support comprising a poly(T) sequence, such that said poly(A) sequence hybridizes with said poly(T) sequence;

c) removing unhybridized first probe sequences;

d) denaturing said first hybridization complex;

e) amplifying said first probe to generate a plurality of amplicons;

f) contacting said amplicons with an array of capture probes to form assay complexes;

and

g) detecting said assay complexes.

2. A method according to claim 1 wherein said first probe comprises a label.

3. A method according to claim 2 wherein said label is a primary label.

4. A method according to claim 3 wherein said primary label is a fluorescent label.

5. A method according to claim 2 wherein said label is a secondary label.

6. A method according to claim 2 wherein said label is biotin.

25 7. A method of detecting a first target sequence comprising a first target domain, a second adjacent target domain and a poly(A) sequence, said method comprising:

a) hybridizing a first probe comprising:

i) an upstream universal priming site (UUP); and

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15. A method according to claim 1 or 7 wherein said amplifying is done by:

- a) hybridizing a first universal primer to said UUP;
- b) providing a polymerase and dNTPs such that said first universal primer is extended;
- c) hybridizing a second universal primer to said DUP;
- d) providing a polymerase and dNTPs such that said second universal primer is extended; and
- e) repeating steps a) through d).

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Sub P3
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16. A method according to claim 1 or 7 wherein said array comprises:

- a) a substrate with a patterned surface comprising discrete sites; and
- b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.

17. A method according to claim 16 wherein said discrete sites comprise wells.

18. A method according to claim 16 wherein said substrate comprises a fiber optic bundle.

19. A method according to claim 1 or 7 wherein said support comprising a poly(T) sequence comprises magnetic beads.

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